FIG._1

FIG._2

FIG._3

FIG._5

FIG._4

BLACK BOX = REGION TO BE MUTATED

F1   F2   F3

TEMPLATE DNA

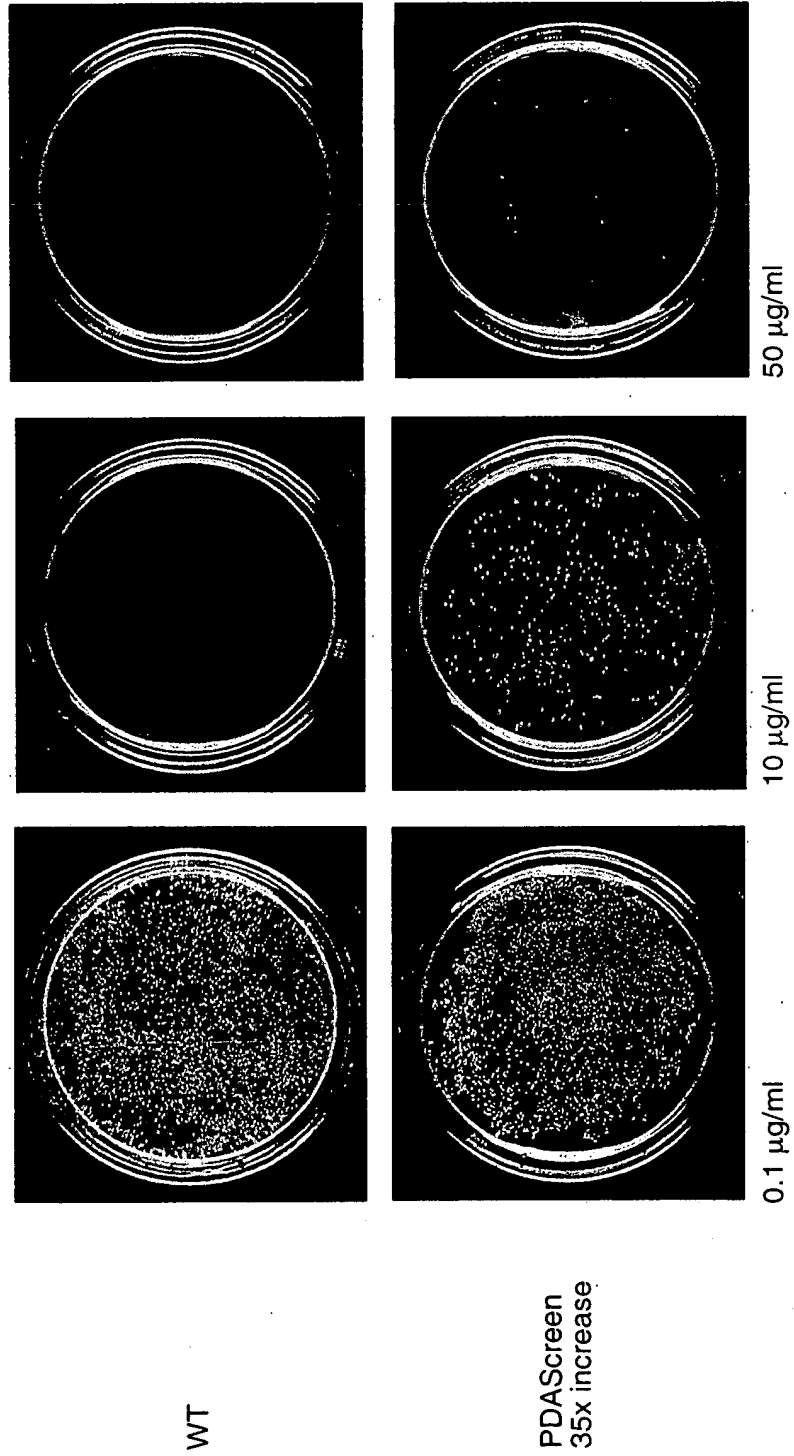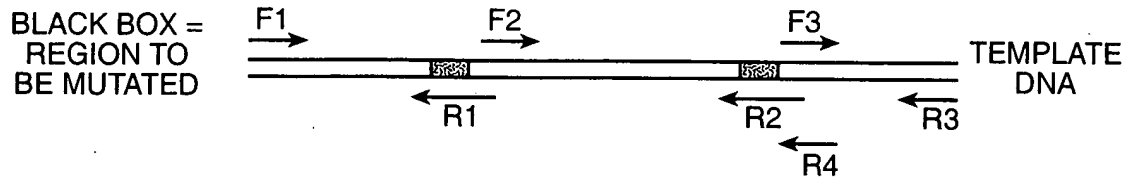R1   R2   R3

R4

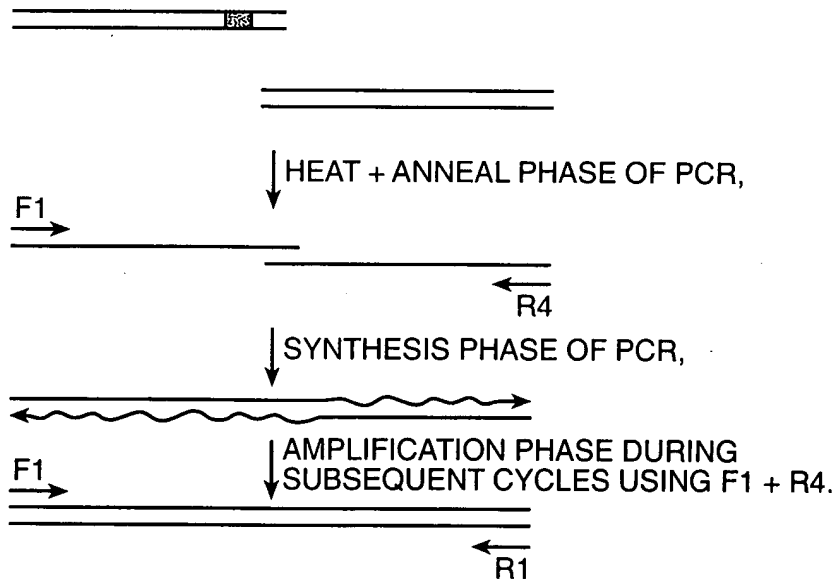STEP 1: SET UP 3 PCR REACTIONS:

PRODUCTS:

TUBE 1:

TUBE 2:

TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.
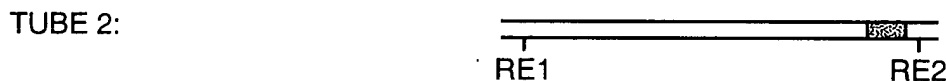
↓ HEAT + ANNEAL PHASE OF PCR,

F1

R4

↓ SYNTHESIS PHASE OF PCR,

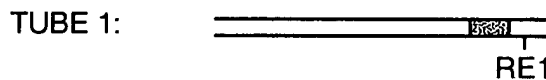↓ AMPLIFICATION PHASE DURING SUBSEQUENT CYCLES USING F1 + R4.

F1

R1

STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG._6

STEP 1: SET UP 3 PCR REACTIONS:
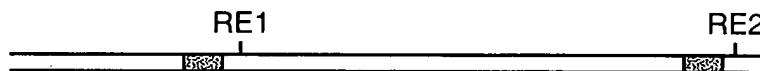
TUBE 1:

TUBE 2:

TUBE 3:
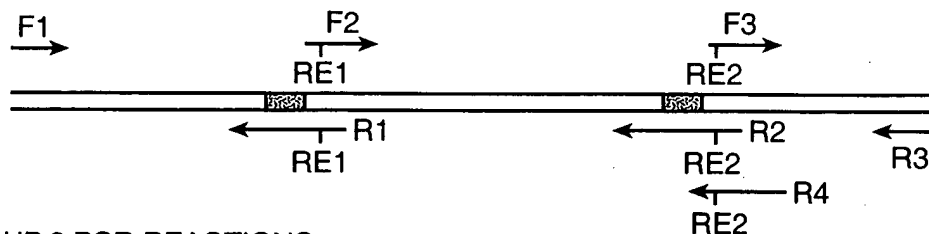
STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE
RESTRICTION ENDONUCLEASES.
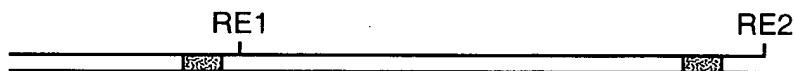
STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH
DIGESTED PRODUCT FROM STEP 2, TUBE 1.

STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.

STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4
WITH RESTRICTION ENDONUCLEASE #2.

STEP 6: LIGATE PRODUCT FROM STEP 5 WITH
PRODUCT FROM STEP 2, TUBE 1.

STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.
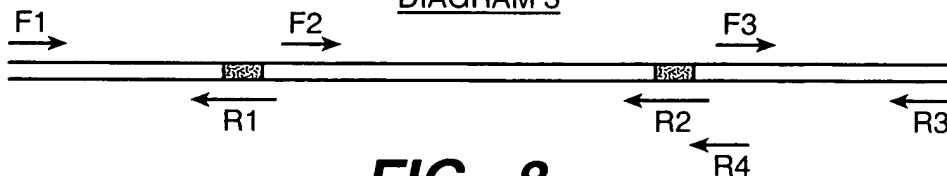
FIG._7

DIAGRAM 3

FIG._8

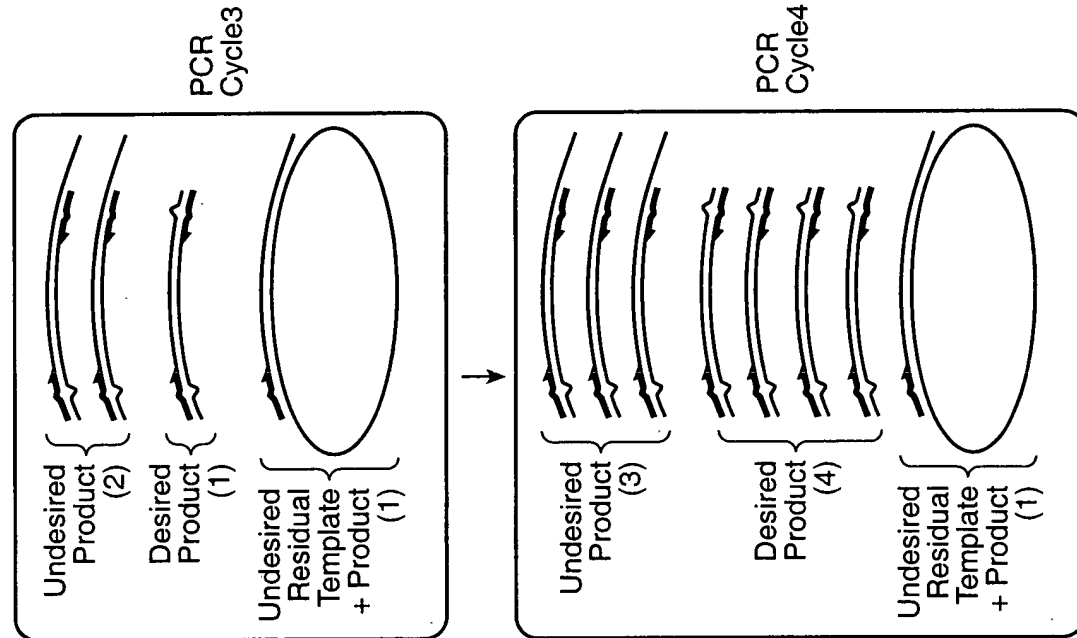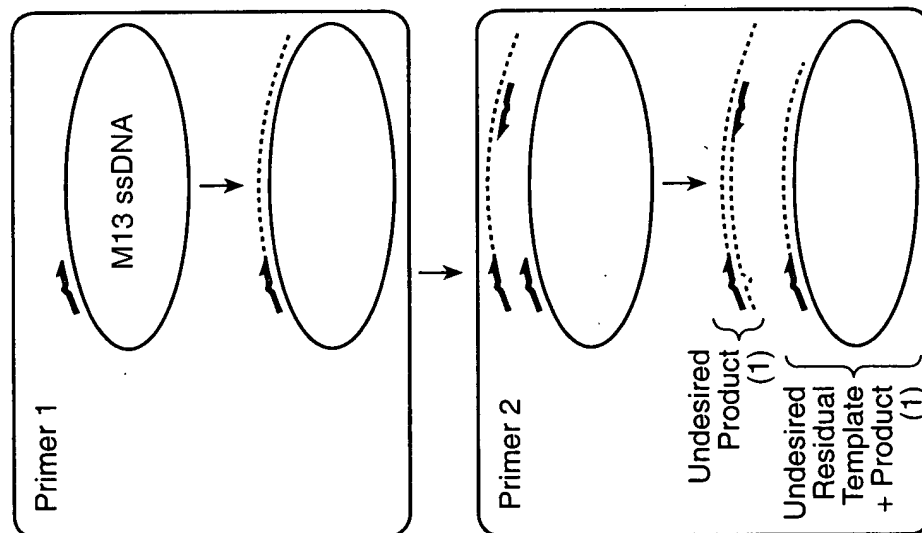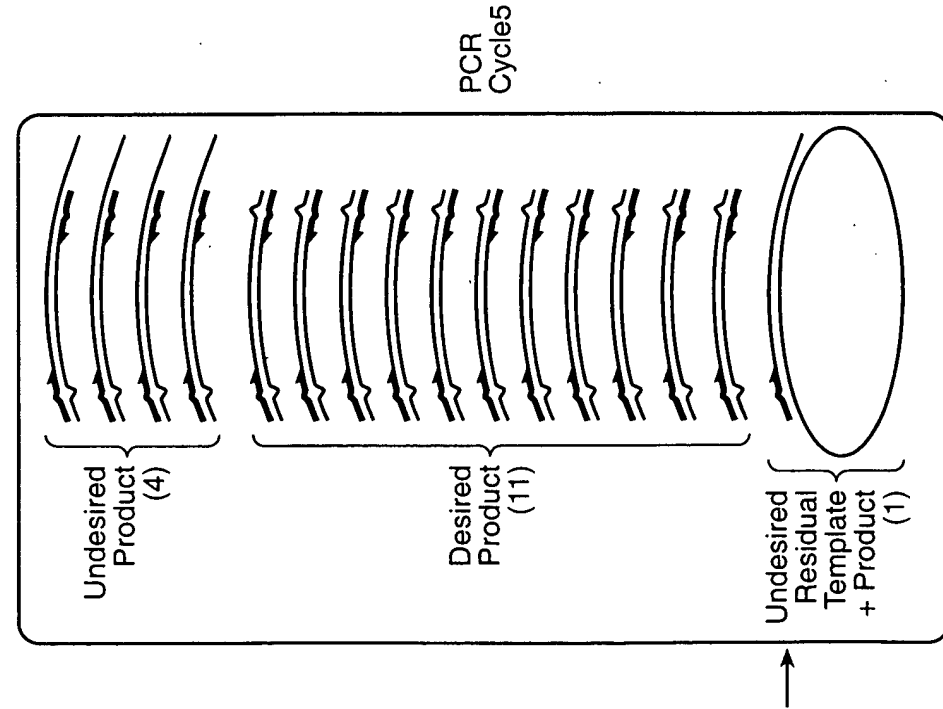Amplification Scheme Based on M13 Single Stranded Template

FIG._9A

FIG._9B

Amplification Scheme Based on M13 Single Stranded Template

Numerical Progression of Desired Product with Increasing PCR Cycles

PCR Cycles	Desired Product	Undesired Products and Residual Template	Percent Desired Product in Total Product
1		1	0.00%
2	0	2	25.00%
3	1	3	50.00%
4	4	4	68.75%
5	11	5	81.25%
6	26	6	89.06%
7	57	7	93.75%
8	120	8	96.48%
9	247	9	98.05%
10	502	10	98.93%
11	1013	11	99.41%
12	2036	12	99.68%
13	4083	13	99.83%
14	8178	14	99.91%
15	16369	15	99.95%
16	32752	16	99.97%
17	65519	17	99.99%
18	131054	18	99.99%
19	262125	19	99.99%
20	524268	20	100.00%

PCR Cycle5

Undesired Product (4)

Desired Product (11)

Undesired Residual Template + Product (1)